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Development of a time-to-positivity assay as a tool in the antibiotic management of septic patients

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ABSTRACT

Optimal use of antibiotics is a key component of the management of sepsis. The purpose of this study was to develop a modification of the time-to-positivity (T_{pos}) assay for use in the acute management of septic patients. Initial laboratory experiments, followed by ex-vivo validation and pilot studies, were performed with a small number of healthy human volunteers and 46 septic patients on a general intensive care unit, chosen on the basis of their antibiotic regimen. The study demonstrated that the T_{pos} assay could be used as a surrogate for antimicrobial activity, and provided preliminary data to demonstrate how this approach might be used to monitor the efficacy of antibiotic therapy in septic patients. The T_{pos} assay might offer a quick and convenient way to improve the efficacy of antibiotic therapy in septic patients, and further prospective large-scale studies are now warranted.

Keywords Antibiotic therapy, infection, patient management, sepsis, therapy, time-to-positivity assay

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INTRODUCTION

Bacterial sepsis remains a leading cause of morbidity, with a particularly high mortality rate among patients in intensive care units (ICUs), and also constitutes an enormous healthcare cost. Antibiotic therapy is a cornerstone of management [1], but until there is microbiological evidence of infection, most patients are initially treated empirically. A considerable body of evidence has shown that *appropriate* antibiotic treatment (i.e., a regimen that is active against the causative pathogen) is associated with a significantly improved outcome [2,3]. The quicker such a regimen is employed, the better the outcome is likely to be. However, conventional microbiological methods typically require that cultures be incubated for 12–18 h before a preliminary diagnosis can be made. Hence, a test that would provide evidence of the adequacy of antibiotic therapy in a shorter period would potentially be of clinical value.

'Time-to-positivity' (T_{pos}) assays depend on the ability of automated blood culture detection systems to 'flag' a sample as soon as it is registered as positive. Whereas conventional blood culture bottles are inspected visually for evidence of bacterial growth perhaps once or twice during initial overnight incubation, the automated systems allow very frequent (sometimes continuous) monitoring, and allow detection of a positive sample much more quickly. Because the density of the inoculum is one of the key variables that determines how quickly a sample will register positive, several investigators have explored the notion that T_{pos} could be used as a *diagnostic* test, particularly for intravascular catheter-associated infections [4,5].

The present study hypothesised that, if a serum sample of a patient being treated for sepsis was inoculated with one or more standard organisms, the T_{pos} could be used as a surrogate marker of total antibacterial activity in the sample, and hence the adequacy of treatment. In simple terms, it might be expected that the longer the T_{pos}, the greater the antimicrobial activity. This article describes and validates the method, and shows how it could be applied to the management of septic patients in an ICU.

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MATERIALS AND METHODS

Bacterial strains, serum and inoculum preparation

This study used clinical isolates from blood cultures of septic patients in an ICU: *Staphylococcus aureus* (methicillin-resistant) (BC 2985), *Escherichia coli* (BC 3164) and *Pseudomonas aeruginosa* (BC 6119). MICs were determined with the Etest method [6]. One antibiotic was tested for each isolate: vancomycin (Lilly, Basingstoke, UK) against *S. aureus*; gentamicin (Roche Diagnostics, Lewes, UK) against *E. coli*; and imipenem (MSD, Hoddesdon, UK) against *P. aeruginosa*. Normal human serum was obtained from a small number of healthy adult volunteers. The sera were stored as aliquots at −20°C.

A standardised inoculum of 0.5×10^6 to 1×10^6 CFU was prepared by inoculating four to five colonies of a pure culture into 5 mL of Mueller–Hinton (MH) broth. The broth was incubated for 2 h at 35°C until it was visibly turbid (in mid-logarithmic phase). The inoculum size was measured with the Miles and Misra method [7].

In-vitro studies

In order to determine whether there was a direct quantitative relationship between the number of bacteria in a blood culture and the T_{pos}, tubes containing 1.8 mL of pooled healthy human serum were inoculated with 0.2 mL of a series of ten-fold dilutions of the standardised inoculum of each of the bacteria in turn. The inoculated sera were transferred into BacT/ALERT bottles (bioMérieux, Durham, NC, USA) for aerobic incubation.

In order to determine whether the T_{pos} was influenced by the addition of antibiotics, tubes containing 1 mL of normal human serum and a range of antibiotic concentrations (covering the normal therapeutic levels) were inoculated with 1 mL of MH broth containing 0.5×10^6 to 1×10^6 CFU for each antibiotic/bacterium pair, and then transferred to BacT/ALERT bottles for aerobic incubation.

Ex-vivo studies

In order to determine the effect on the T_{pos} as the antibiotic was eliminated, a single therapeutic dose of antibiotic was injected intravenously into a healthy volunteer and serial blood samples were taken at various time-points thereafter. Serum was separated from the samples, and 1-mL aliquots were inoculated with 1 mL of MH broth containing 0.5×10^6 to 1×10^6 CFU; the mixtures were then transferred into BacT/ALERT bottles for aerobic incubation.

In order to estimate the variability of the T_{pos} in clinical samples from septic patients, random blood samples were taken from 46 patients in the ICU who were being treated with the selected antibiotics. Serum was separated from the samples, and 1-mL aliquots were inoculated with 1 mL of MH broth containing 0.5×10^6 to 1×10^6 CFU; the mixtures were then transferred into BacT/ALERT bottles for aerobic incubation. The microorganism tested was selected to match the antibiotic that the patient was receiving. A positive control (microorganism plus a known concentration of antibiotic) and a negative control (microorganism alone) were included.

All experiments involving healthy human subjects or patients were carried out with the approval of the Hammersmith Hospitals Trust Research Ethics Committee.

Statistical methods

Data were entered into the SigmaStat program (SPSS Inc., Chicago, IL, USA), and linear correlation coefficients were computed. Where necessary, the values were log-transformed before calculation.

RESULTS

The MIC of vancomycin for the *S. aureus* isolate was 4 mg/L, the MIC of gentamicin for the *E. coli* isolate was 1 mg/L, and the MIC of imipenem for the *P. aeruginosa* isolate was 4 mg/L.

There was a linear correlation between the number of CFU inoculated into healthy human serum and the T_{pos} in the Bact/Alert system, even at very low numbers (0.93×10^1 CFU) (Fig. 1). This relationship was consistent and reproducible for each antibiotic–microorganism combination tested. There was a good correlation between the T_{pos} and increasing concentrations of antibiotic in healthy human serum inoculated with a fixed number of bacteria (Fig. 2). However, for the combinations *S. aureus*–vancomycin and *P. aeruginosa*–imipenem, the relationship was not seen for the lowest concentrations of antibiotic.

The natural pharmacokinetic decay of antibiotic concentration in the serum of a healthy subject was reflected in the T_{pos} when the serum was inoculated with a fixed number of bacteria. As the antibiotic was cleared, the T_{pos} shortened (Fig. 3), demonstrating that the T_{pos} could be used as a surrogate for antimicrobial activity.

Random serum samples, obtained from patients in the ICU, that were spiked with test organisms showed a range of T_{pos} values. The negative controls provided the baseline, and the positive controls fell within the range of the results from patients. There was sufficient variability in the T_{pos} values in clinical samples from patients receiving antibiotic therapy to ensure discrimination between high and low values, within the limits of the assay (Fig. 4).

DISCUSSION

The importance of optimising antibiotic therapy in septic patients is widely accepted. A large number of clinical investigations have shown that the mortality rate in patients treated with appropriate antibiotics is lower than in those not receiving appropriate antibiotics [3,8]. In this

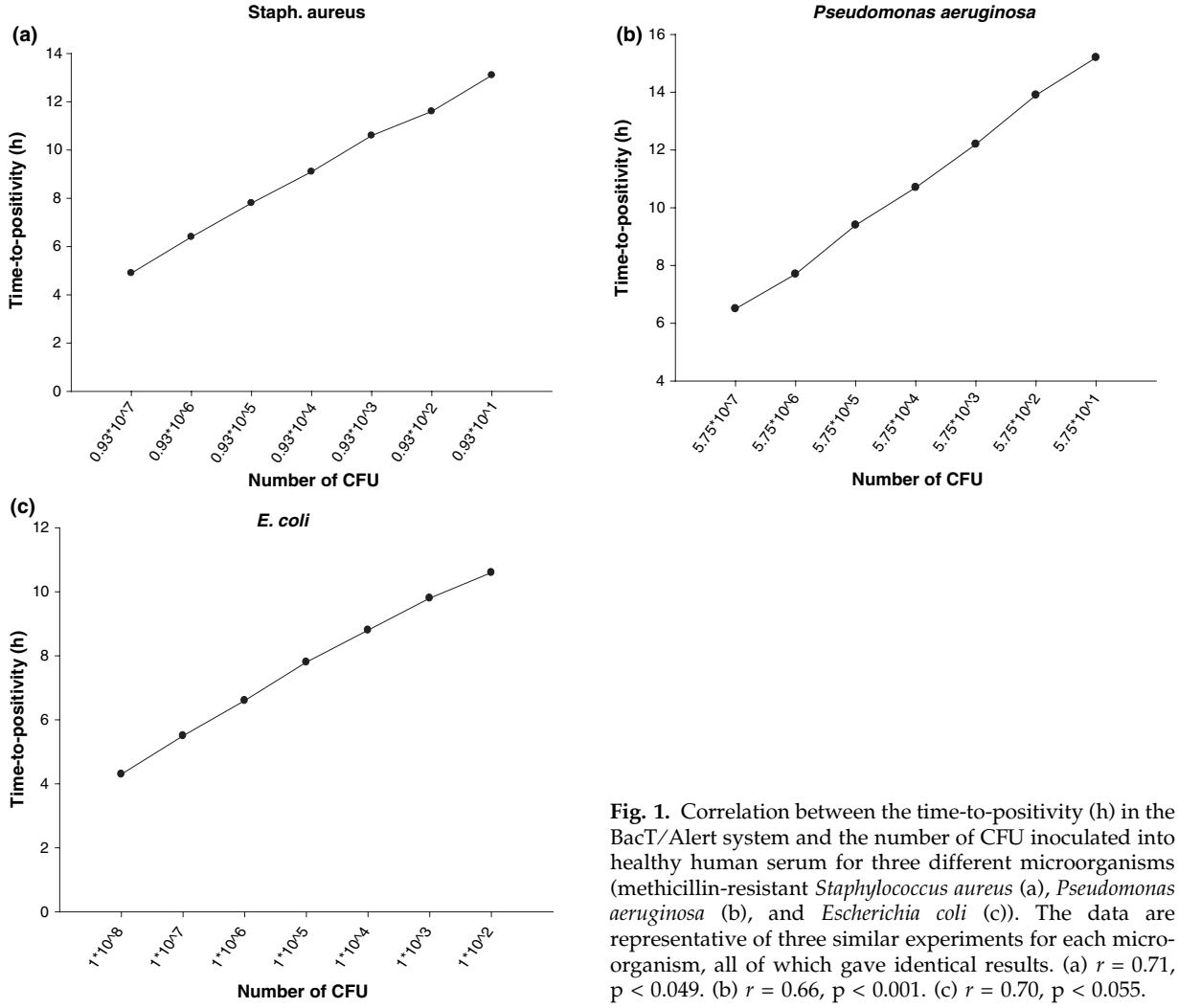

Fig. 1. Correlation between the time-to-positivity (h) in the BacT/Alert system and the number of CFU inoculated into healthy human serum for three different microorganisms (methicillin-resistant *Staphylococcus aureus* (a), *Pseudomonas aeruginosa* (b), and *Escherichia coli* (c)). The data are representative of three similar experiments for each microorganism, all of which gave identical results. (a) $r = 0.71$, $p < 0.049$. (b) $r = 0.66$, $p < 0.001$. (c) $r = 0.70$, $p < 0.055$.

context, 'appropriate' is usually taken to mean an antibiotic that is active *in vitro* against the organism causing the infection. However, truly appropriate antibiotic therapy goes beyond simple in-vitro activity, since it is also dependent on pharmacokinetic and pharmacodynamic considerations that relate to tissue distribution and the activity of an antibiotic in a clinical environment that is often far removed from the ideal conditions in which pharmacological studies are performed.

The serum bactericidal assay (or serum bactericidal test, or titre; SBT) ought to be the ideal way to assess the appropriateness of antibiotic therapy, since it is one of the few ex-vivo tests performed in the clinical laboratory that reflects the interaction of the isolated pathogen, the antimicrobial agent and the patient. However, most investigators regard the SBT as imprecise and time-consuming, and because quality control schemes have shown it to have poor reproducibility, it has generally fallen into disuse.

The present article describes a novel approach that extends the principle of T_{pos} assays from use in diagnosis to use in patient management. The key findings are (1) that T_{pos} correlates directly with antimicrobial activity in serum, both in-vitro and ex-vivo, and (2) that measurements of T_{pos} in septic patients in an ICU show considerable differences, suggesting that there is indeed significant variability in the antimicrobial activity of common antibiotic regimens, and hence an opportunity to use this method to optimise therapy in these very ill patients.

A number of potential limitations to this approach must be recognised. The method

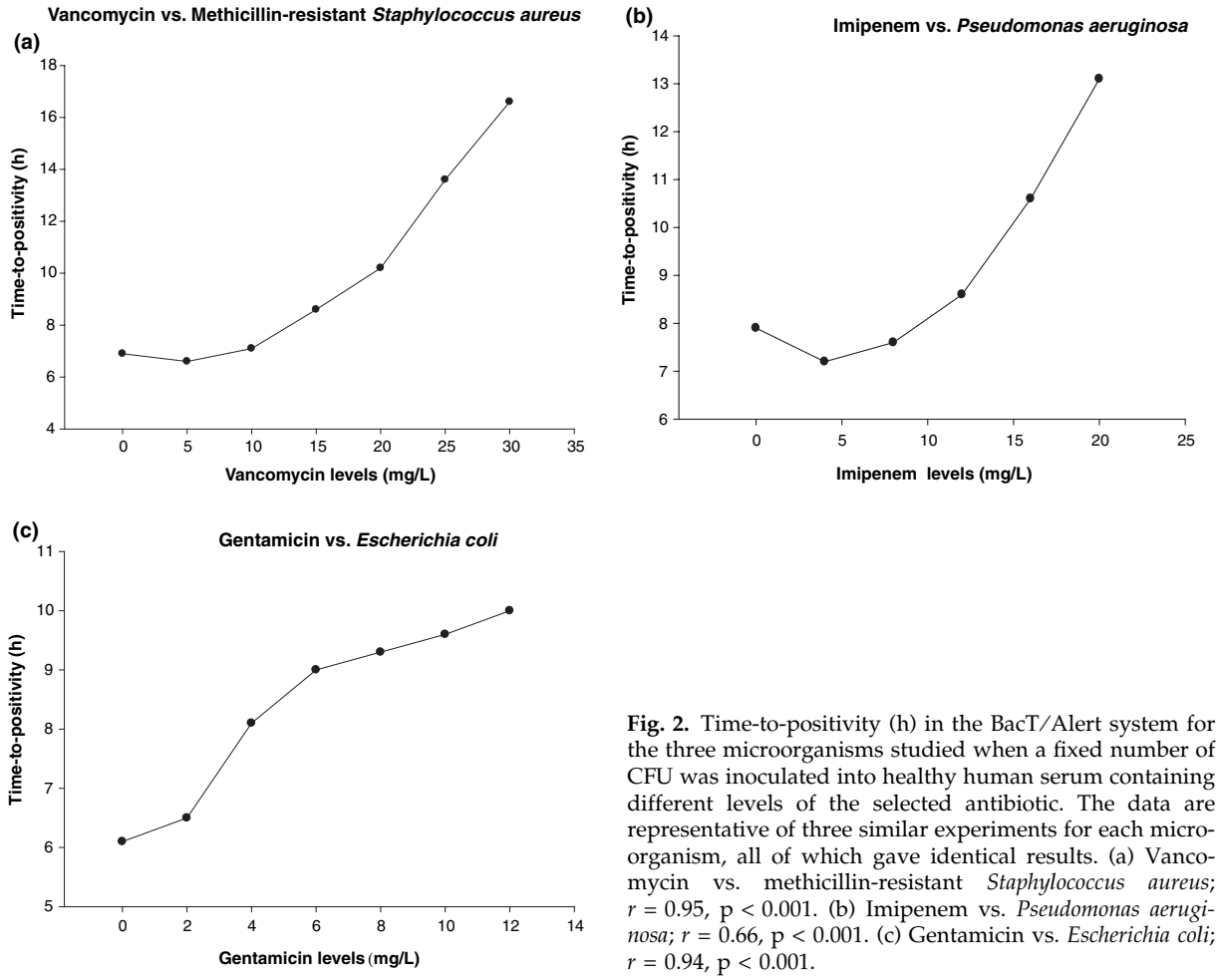

Fig. 2. Time-to-positivity (h) in the BacT/Alert system for the three microorganisms studied when a fixed number of CFU was inoculated into healthy human serum containing different levels of the selected antibiotic. The data are representative of three similar experiments for each microorganism, all of which gave identical results. (a) Vancomycin vs. methicillin-resistant *Staphylococcus aureus*; $r = 0.95$, $p < 0.001$. (b) Imipenem vs. *Pseudomonas aeruginosa*; $r = 0.66$, $p < 0.001$. (c) Gentamicin vs. *Escherichia coli*; $r = 0.94$, $p < 0.001$.

involves the assumption that the patient does indeed have a bacterial infection that requires antibiotic therapy. If the patient is not infected, then the test will yield a 'false-positive' result, in that there may well be antibiotic activity in the patient's serum, directed against the control organism. Similarly, the result will be misleading if the patient is infected with a fungus. Both of these limitations reflect a general clinical requirement that a test must be interpreted in the context of a correct underlying diagnosis. It also needs to be acknowledged that, at present, this approach would only be valid for the *c*. 20–25% of septic patients who are bacteraemic. Future studies should evaluate patients with a focus at a different site.

A second potential problem is that the bactericidal activity demonstrated against the control organism might be very different to that against the actual infecting pathogen. The control organisms used were chosen to represent typical ICU pathogens, but the confidence with which a result can be extrapolated from a control organism to a patient's isolate remains unknown until further, more extensive experience has been gained. An alternative approach would involve performing the assay with the patient's isolate as soon as it is available. This would have the advantage of greater specificity, but the disadvantage of introducing a delay before the test could be performed. Future studies should compare these two approaches to determine which yields the more useful results.

Finally, a concern was that the method would not be sufficiently robust or reproducible to be clinically useful, or that the degree of variability in the T_{pos} in patients in an ICU would be insufficient to make measurement worthwhile. However, the initial data suggest that the results are reproducible and that there are sufficiently large differences in the clinical measurements to justify a prospective study comparing T_{pos} with

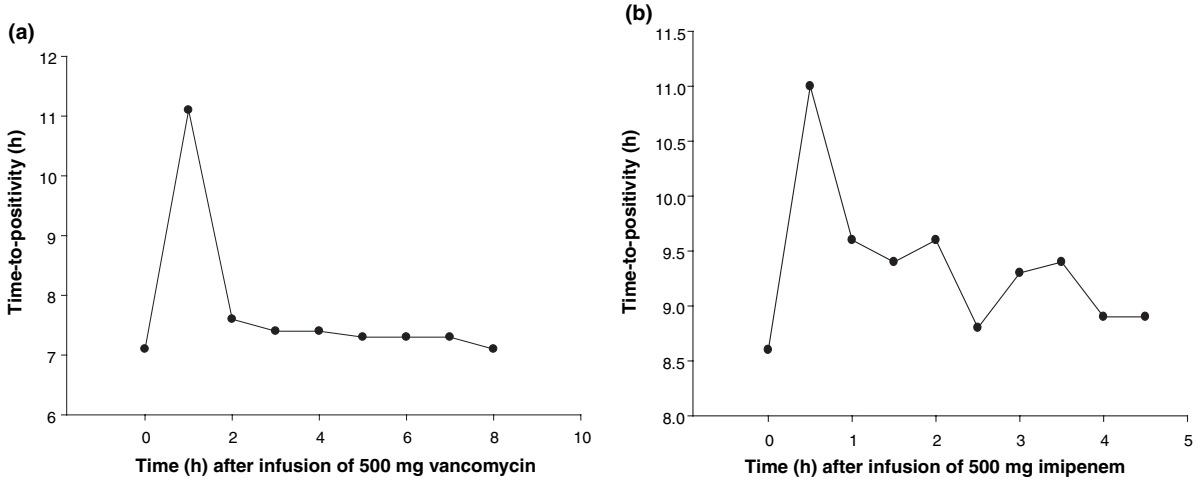

Fig. 3. Time-to-positivity (h) in serial blood samples obtained after infusion of a single dose of (a) vancomycin or (b) imipenem in a healthy volunteer. Each sample was inoculated with a fixed number of CFU (methicillin-resistant *Staphylococcus aureus* for vancomycin, and *Pseudomonas aeruginosa* for imipenem).

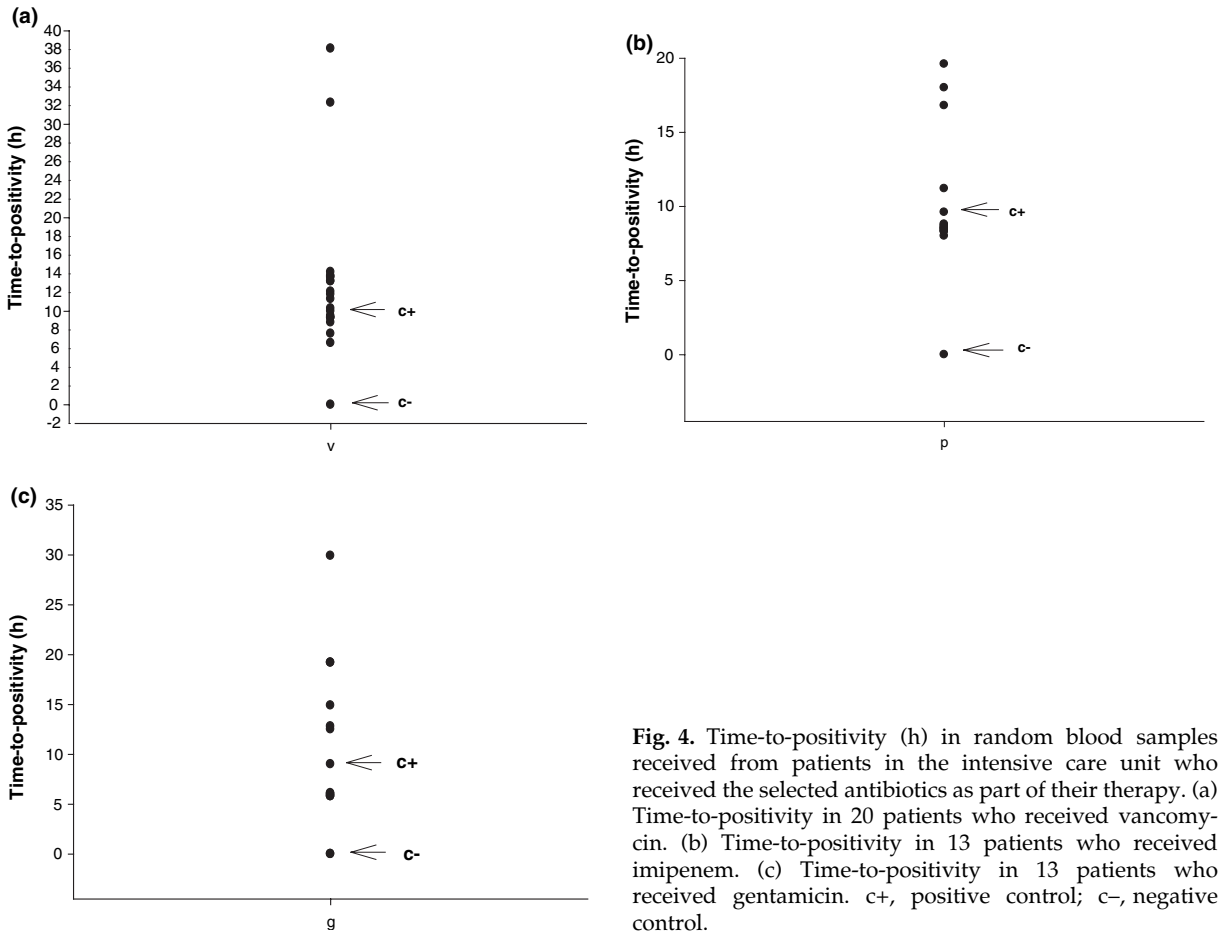

Fig. 4. Time-to-positivity (h) in random blood samples received from patients in the intensive care unit who received the selected antibiotics as part of their therapy. (a) Time-to-positivity in 20 patients who received vancomycin. (b) Time-to-positivity in 13 patients who received imipenem. (c) Time-to-positivity in 13 patients who received gentamicin. c+, positive control; c–, negative control.

clinical outcomes. Preliminary data (not shown) suggest that there is indeed a correlation between T_{pos} and length of stay in the ICU.

Optimising antibiotic therapy for septic patients in the ICU is an important goal. The method described in this paper is attractive

because it is less technically demanding than the standard SBT assay, and requires only one additional blood sample. Further studies are warranted to establish whether this approach may be of value in monitoring antibiotic therapy in individual patients, and/or as a method to evaluate the efficacy of various antibiotic strategies in defined patient groups.

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